ATTACHMENT F

CBNP Detection Thrust Area

QUARTERLY PROGRESS REPORT FOR THE PERIOD [redacted]

PROJECT TITLE: CBNP Detection Thrust Area
DOE/HQ PROJECT NUMBER: CB04LL **LAB/CONTRACTOR:** LLNL
B&R CODE: GC0404 **DATE:** [redacted]
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PROGRESS (BY TASK) DURING QUARTER:

Task 1. The Autonomous Pathogen Detector System (APDS)
The Autonomous Pathogen Detector System (APDS) Project is focused on the development of a stand alone instrument that provides automated continuous monitoring for BW agents at special events or locations. The APDS's functionality includes continuous or on-demand aerosol sampling, sample preparation, automated fluidic sample handling and transport, detection/identification by a combination of flow cytometry immunoassay and nucleic acid recognition (PCR) and automated data analysis and reporting. The primary technical objectives in FY[redacted] are to evaluate and demonstrate several major hardware components that build on the first version instrument, the APDS-I, for integration into the next generation instrument, the APDS-II. Our progress during the first quarter is summarized below.

aerosol collector
We are currently evaluating three different aerosol collectors for use in the APDS-II. They include the SASS-2000 (Smart Air Sampler System, Research International), a hybrid LLNL virtual impactor–SASS-2000, and the SCAEP (Space Charged Atomizing Electrostatic Precipitation, Team Technologies). Side-by-side system performance comparisons of these different units will be performed in FY[redacted] at the Harry Reid Center (HRC) for Environmental Studies, University of Nevada-Las Vegas (UNLV). Of particular interest in the operation of these aerosols is collection efficiency. The collector with the best overall performance will be integrated into the APDS-II architecture in FY[redacted].

- Wrote an experimental test plan in collaboration with Dr. Linda Stetzenbach of the Harry Reid Center for Environmental Studies, University of Nevada-Las Vegas (UNLV) for testing the three aerosol collectors at UNLV. Details have been finalized for testing the collectors using B.g. spores. An experimental chamber measuring 4.0 m x 4.0 m x 2.2 m will be used to conduct the experiments. A well controlled particle size distribution of dry B.g. spores obtained from Dugway Proving Grounds will be introduced into the experimental room via an air supply handling unit. A laser-based aerodynamic particle sizer placed inside the chamber will measure real-time particle concentration as a function of size. A referee aerosol collector will be provided by UNLV as part of the intercomparison. The liquid collection buffer in each sampler will be analyzed at the HRC laboratory using culture-based analysis, quantitative

polymerase chain reaction and electronic particle counting. In addition, 2-3 ml aliquots of the liquid collection material, both background and aerosolized B.g. will be archived and prepared for transport to LLNL for analysis. Tests will begin in late [redacted] and run for approximately four-six weeks at a cost of $10,000.

- Completed preparations for each aerosol collector prior to shipment to UNLV. These preparations included implementing electronic control, system cleaning, and characterization of background collections in the laboratory.

- Shipped aerosol collectors to UNLV in December.

fluidics module

We are currently evaluating two approaches for the APDS-II fluidic system. The key requirements for this system are to provide small reaction volumes necessary for the Luminex cytometer, and to provide sample clean-up steps to enhance the performance and sensitivity of the multiplex bead assays.

- A flow-through filtration system has been developed and incorporated in the fluidic system used for APDS-I to test this approach for improving assay sensitivity. The existing breadboard system has been reconfigured to accommodate the new sample preparation protocol for the Luminex, model LX-100. The major change was to add a sequential addition of reagents with a filtration step following each addition. The software is in the process of being altered to accommodate this enhancement.

- We have also designed an alternative approach to the fluidic system using syringe pumps and multi-port valves. A prototype system is currently being fabricated by Global-FIA at a cost of $10,000. Delivery of this device is expected in [redacted]

- The prototype flexible plastic fluidic system has been assembled and is ready for testing. The apparent need of a filtration step to enhance assay performance complicates the utilization of this approach. We are therefore currently pursuing the investigation of the two methods described above over this approach.

multiplex flow cytometry

A major advancement related to large-scale multiplex immunoanalysis is the recent release of the Luminex, model LX-100 portable flow cytometer. This instrument was not available when we designed and built the APDS-I. The LX-100 constitutes the centerpiece of our development effort for incorporating multiplex flow cytometry into the APDS-II system. However there are a number of technical challenges that need to be addressed in order to appropriately apply this technology to our detection system. Specific areas for development include instrument integration, assay development, enhanced assay capabilities, and extended multiplexing.

- Luminex has provided a complete optical and fluidic upgrade for the LX-100 flow cytometer that was completed in [redacted] The bead sets we have been previously using for assays are not compatible with this upgrade, however we have obtained new bead sets that are compatible with the recent upgrade.

- We have coupled simmulant antibodies to the new bead sets and are assessing the performance of the modified optics of the LX-100 with multiplex immunoassays.

- Aerosol collector fluid samples containing background particulates have been acquired to test the effect of these particulates on assay performance.

flow-through PCR

The addition of PCR to the antibody assays is a critical step to increasing the reliability of positive identifications and to provide detailed analysis of the origin and characteristics of detected pathogens. Flow-through PCR was not included in the APDS-I instrument but is planned to be integrated into APDS-II as an add-on capability in FY01. *Multiplex* flow-through PCR will be demonstrated in FY[redacted] and fully integrated in the APDS-III instrument in FY[redacted]

- The fluidics of the current flow through PCR prototype have been modified to increase the stability of sample delivery and allow sequential PCR analyses.

- Modifications to the thermal cycling profiles have been developed to demonstrate successful PCR identification of three bacterial species.

system integration of the APDS-II instrument

As each of the various hardware elements (for example, aerosol collector, fluidics module, control system, LX-100 cytometer, etc.) of the APDS-II evolve, it will be necessary to integrate these components into a rugged, autonomous detection system.

- Developed a specifications document that describes each major component with specifications for the APDS-II instrument. This document is considered a 'living document' in that it will constantly be reviewed and updated as components evolve and technical progress is made.

- Completed a preliminary design of the APDS-II instrument controls, and display functions. This task requires that we closely track the progress of the Luminex, LX-100 model control software so that the eventual interface between the APDS-II control system and the LX-100 goes smoothly.

Task 2. MEMS Fluidics Module (MFM)

We are building an integrated biological processor based on novel MEMS (Micro Electro Mechanical Systems) components to yield a compact, rugged, low power unit capable of both immuno- and DNA-based assays. This module will be fabricated using standard and novel micromachining techniques using inexpensive plastic materials. The components to be incorporated into this module include: an acoustic fractionator, an acoustic mixer for mixing the antibody-coated beads and sample, utilization of dielectrophoretic concentration and purification of biological particles. MHD (magnetohydrodynamic) pumps will be used to pump and switch fluids throughout the device without using moving parts. In order to satisfy the challenging demands of the APDS, this subsystem will incorporate such functions as preconcentration, purification, lysing, mixing, pumping, and reconstitution of reagents. This module will be incorporated into the final

version of the APDS (APDS-III) instrument. We are also investigating the feasibility of adding a detection capability directly on-board the MFM module.

materials and process

- The material of choice for our system is acrylic. This material was chosen primarily for its acoustic properties and because it is used for many commercial microfluidic devices.

- Processing techniques were developed for this material including etching, bonding and metalization. While some refinement of these processes is required, they are well enough in hand to build prototype parts.

dielectrophoretic(DEP) concentration and purification

- Prototype dielectrophoretic concentrators have been designed using the acrylic process. Fabrication will proceed once the masks arrive.

magnetohydrodynamic (MHD) pumping

- Prototype MHD switches have been built and verified. The pressure head supplied by the pumps is lower than desirable. New magnets are being designed to increase the head pressure by an order of magnitude.

acoustic mixing

- Prototype acoustic mixers have been manufactured and are currently being tested. The next step will be to check the antibody-antigen binding efficiency.

system design

- The overall system design is proceeding with a preliminary device design that incorporates the above components and will demonstrate an advanced immunoassay sample preparation system.

COMMENTS: NONE

FUNDING STATUS (BY TASK):

	OPER $K	CAP $K
FY[redacted] CARRYOVER:	$60.4	$0
FY[redacted] FUNDING:	$4,555.	$0
TOTAL FY00 FUNDING AVAILABLE:	$4,615.4	$0
$ SPENT THIS QUARTER:	$669	$0
$ SPENT YEAR-TO-DATE:	$669	$0
$ REMAINING FOR THIS FY:	$3,946.4	$0
$ LIENS	$45	$0
ANTICIPATED UNCOSTED CURRENT FY FUNDS:	$0	$0

TECHNICAL REPORTS/PRESENTATIONS:

Hosted 1st Quarter-FY[redacted] CBNP Quarterly Review Meeting at LLNL, [redacted]

"Autonomous Pathogen Detector," Rich Langlois, 1st Quarter-FY[redacted] CBNP Quarterly Review Meeting, LLNL, [redacted]

"Autonomous Pathogen Detector," Robin Miles, 1st Quarter-FY[redacted] CBNP Quarterly Review Meeting, LLNL, [redacted]

"Biological Sampling Efficiency of the Hybrid LLNL Virtual Impactor/Research International's SASS 2000 Biocollector," Steve Brown, LLNL, and Linda Stetzenbach, University of Nevada-Las Vegas, [redacted]

"Autonomous Pathogen Detection System (APDS-II) Specification Document, version 1.1,) Bill Colston, LLNL. [redacted].

"APDS Lifecycle Report," Rich Langlois, Robin Miles and Al Ramponi, submitted to the Detection Area Thrust Leader (John Vitko, SNL) as part of the FY[redacted] Lifecycle Plan to DOE, [redacted].

"The Autonomous Pathogen Detector System (APDS)," Rich Langlois, submitted for DOE Annual Report, [redacted].

"Autonomous Biodetector," Rich Langlois, NAI Advisory Committee Review, LLNL, [redacted].

"MEMS-based Sample Preparation," Robin Miles, NAI Advisory Committee Review, LLNL, [redacted].